In re Application of:

Shamblott, et al.

Application No.: 09/767,421 Filed: January 22, 2001

Page 2

PATENT Attorney Docket No.: JHU1750-1

Amendments to the Claims

Please amend claims 1, 9-13, 15, 22-23, 26 and 32 as indicated in the listing of claims.

Please cancel claims 2-8 and 17-21.

Claims 14 and 33 were previously canceled and withdrawn, respectively.

The listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Currently amended) [[A]] An enriched cell culture population of cells comprising, a human embryoid body derived (EBD) cells, wherein the cells are characterized by not causing formation of a teratoma when injected into having teratogenic properties in SCID mice[[,]], and and wherein at least some of the cells simultaneously express express at least a first and a second polypertide or mRNA marker markers that are characteristic of from at least two

second polypeptide or mRNA marker markers that are characteristic of from at least two different cell types, wherein the cell types are selected from ectodermal cells, a mesodermal cells, or endodermal cells, and wherein the first marker is selected from the group consisting of nestin, vimentin, neurofilament light isoform, microtubule-associated protein 2c, tau, nonphosphorylated neurofilament heavy isoform, neuron-specific enolase, tyrosine hydroxylase, glial fibrillary acidic protein, CNPase, and galactocerebroside, and the second marker is selected from the group consisting of myf-6, myosin light-chain 2 ventricular isoform, flk1, α -1-fetoprotein, and GATA-4.

2-8. (Canceled)

9. (Currently amended). The <u>culture cells</u> of claim 1, <u>wherein that</u> under suitable cell culture conditions <u>proliferates</u> the <u>cells proliferates</u> for at least thirty population doublings without being immortal under said conditions.

In re Application of:

Shamblott, et al.

Application No.: 09/767,421

Filed: January 22, 2001

Page 3

10. (Currently amended) The culture of claim 9 cells of claim 1, wherein that under suitable cell culture conditions proliferates the cells proliferate for at least sixty population doublings.

PATENT

Attorney Docket No.: JHU1750-1

- 11. (Currently amended) The culture of claim 1 cells of claim 1, wherein that proliferates the cells proliferate under cell suitable cell culture conditions that are nonpermissive for proliferation of human embryonic germ cells.
- 12. (Currently amended) The culture of claim 11 cells of claim 1, wherein that proliferates the cells proliferate in a media under suitable cell culture conditions that lacks lacking leukemia inhibitory factor, a fibroblast feeder layer, or both.
- 13. (Currently amended) The <u>culture cells</u> of claim 1, wherein <u>said</u> the cells are transfectable with a retrovirus or a lentivirus or both.
 - 14. (Canceled)
 - 15. (Currently amended) The culture cells of claim 1, wherein that is the cells are clonal.
- 16. (Currently amended). The culture of claim 15, wherein that is the cells are clonally derived from a single embryoid body derived (EBD) cell.
 - 17-21. (Canceled).

In re Application of:

Shamblott, et al.

PATENT

Attorney Docket No.: JHU1750-1

Application No.: 09/767,421 Filed: January 22, 2001

Page 4

22. (Currently amended) A method of making a human EBD cell-culture an enriched population of cells comprising:

- (a) culturing human embryonic germ primordial germ cells under conditions that are suitable for formation of cystic embryoid bodies,
 - (b) dissociating the cystic embryoid bodies to provide a constituent cell, and
- (c) culturing the constituent cell under conditions suitable to produce a human EBD cell eulture population of cells in serum, reduced serum or serum-free media and further comprising at least some cells in the culture which simultaneously express a first and a second polypeptide or mRNA markers marker that are is characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell, and wherein the first marker is selected from the group consisting of nestin, vimentin, neurofilament light isoform, microtubule-associated protein 2c, tau, nonphosphorylated neurofilament heavy isoform, neuron-specific enolase, tyrosine hydroxylase, glial fibrillary acidic protein, CNPase, and galactocerebroside and the second marker is selected from the group consisting of myf-6, myosin light-chain 2 ventricular isoform, flk-1, α-1-fetoprotein and GATA-4.
- 23. (Currently amended) The method of claim 22 comprising selecting a single EBD cell from the EBD cell culture and culturing the single EBD cell to produce a clonal EBD cell culture population of cells.
- 24. (Original) The method of claim 22 comprising culturing the constituent cell in a media comprising human basic fibroblast growth factor.

In re Application of:

Shamblott, et al.

Application No.: 09/767,421

Filed: January 22, 2001

Page 5

25. (Previously presented) The method of claim 24 comprising culturing the constituent cell in a media selected from the group consisting of RPMI 1640 supplemented with 15% FCS and media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B, fetal

PATENT

Attorney Docket No.: JHU1750-1

bovine serum, VEGF, hFGF, heparin, recombinant human IGF-1 and ascorbic acid.

26. (Currently amended) The method of claim 25 comprising culturing the constituent cell in a media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B, fetal

bovine serum, VEGF, hFGF, heparin, recombinant human IGF-1 and ascorbic acid.

27. (Original) The method of claim 22 comprising culturing the constituent cell on a

matrix.

28. (Original) The method of claim 27 comprising culturing the constituent cell on a

matrix that is selected from the group consisting of collagen I, human extracellular matrix, and

tissue culture-treated plastic.

29. (Original) The method of claim 28 comprising culturing the constituent cell on a

matrix selected from the group consisting of collagen I and human extracellular matrix.

30. (Original) The method of claim 22 comprising culturing the constituent cell on a

media that is not permissive for proliferation of the EG cells.

31. (Original) The method of claim 30 comprising culturing the constituent cell on a

media lacking leukemia inhibitory factor, a fibroblast feeder layer, or both.

32. (Currently amended) The method of claim 22 comprising culturing the EBD cell

culture population of cells for at least 30 population doublings.

GT\6463892.1 331323-80 In re Application of: Shamblott, et al.

Application No.: 09/767,421

Filed: January 22, 2001

Page 6

33. (Withdrawn) A method of treating a human disease or injury comprising introducing a composition comprising an EBD cell or EBD cell culture into the body of a patient having the disease or injury.

PATENT

Attorney Docket No.: JHU1750-1